ACTION OF CLAVACIN ON SOME DERMATOPHYTES*

Final Report†

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This study is concerned with the action of clavacin on the following dermatophytes: Trichophyton gypseum, Trichophyton purpureum, Microsporon audouini, and Monilia albicans. Clavacin is an antibiotic produced by Aspergillus clavatus and by Penicillium patulum, Penicillium claviforme, Penicillium expansum, Aspergillus giganteus, Gymnoascus and other fungi. Synonyms for clavacin are patulin, clavatin and claviformin. In our preliminary report we referred to the pertinent literature (1). Two more papers, dealing with the action of clavacin upon dermatophytes, have to be added to this list, that of Reilly, Schatz and Waksman (2), and that of Hopkins and coworkers (3). We have, subsequent to our preliminary report, repeated our tests with crystalline clavacin against Trichophyton gypseum and Microsporon audouini because of some variations in results which occurred with these fungi.

MATERIALS

In the beginning of this study we prepared a culture filtrate from Aspergillus clavatus, Waksman strain 129, which is known to produce a high yield of a potent clavacin.

The fungus was grown on a synthetic glucose-nitrate medium (Czapek-Dox). It contains 3 Gm. $NaNO_3$, 40 Gm. glucose, 1 Gm. KH_2PO_4, 0.5 Gm. KCl, 0.5 Gm. $MgSO_4 \cdot 7H_2O$, and 0.01 Gm. $FeSO4 \cdot 7H_2O$ in 1000 ml. of tap water. The medium was placed in penicillin flasks, inoculated and incubated at 28°C. On the seventh day the surface growth was removed and the liquid residue was passed through a Seitz filter. The filtrate was stored in the refrigerator.

At a later stage of our work a sample of crystalline clavacin was furnished us. It also was stored in the refrigerator and immediately before each experiment measured quantities were dissolved in sterile distilled water. Clavacin is thermostable, heating at 100° C. for 10 minutes does not reduce its activity.

The test organisms were M. audouini, T. gypseum-granular type, T. purpureum, and M. albicans, all of them freshly isolated from active cases. They were grown and transplanted, at intervals of from one to three weeks, on Sabouraud dextrose agar slants (Difco).

METHODS

A. Experiments with the culture filtrate

A suspension of the test fungus was prepared as follows: 10 ml. of distilled sterile water was poured over a 21 day old culture which did not show signs of

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pleomorphic degeneration. The entire surface growth was scraped off the slant and by agitation thoroughly mixed with the sterile water. Four ml. of the suspension of spores and mycelial fragments were pipetted into a sterile test tube containing 10 ml. of the culture filtrate. As a control 4 ml. of fungous suspension were mixed with 10 ml. of sterile distilled water in another test tube. One loopful of the filtrate-fungus mixture was inoculated on each of three glucose-peptone agar slants at the following intervals: 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 3½ hours or 4 hours, 24 hours or 48 hours. A loopful of the control suspension was inoculated on each of two glucose-peptone agar slants at the same time intervals.

The inoculated slants were kept at room temperature and observed for evidence of fungous growth, at average intervals of three days, for at least three weeks. Growth covering up to one quarter of the surface of the slant was designated as one plus; colonies spreading and covering up to one half of the surface of the slant were designated as two plus; growth covering about three quarters of the surface of the slant was called three plus, and involvement of the entire surface was designated as four plus.

One test was made with the culture filtrate against M. albicans and M. audouini, and two tests against T. gypseum and T. purpureum.

B. Experiments with crystalline clavacin

The crystalline clavacin was dissolved in sterile distilled water. The same procedure was then followed as with the filtrate. The test fungous suspensions were exposed to the following dilutions of clavacin: 1:100 (1%), 1:1000 (0.1%), 1:10,000 (0.01%), 1:100,000 (0.001%), and 1:1000,000 (0.0001%). Four tests were made against M. audouini and T. gypseum, two tests against T. purpureum and one test against M. albicans.

Results

Table 1 shows the fungicidal[1] effect of the undiluted culture filtrate. M. albicans was not demonstrably influenced. T. gypseum was killed in 48 hours, T. purpureum in 90 minutes, and M. audouini in one hour.

Table 2 presents the figures obtained with crystalline clavacin. Although the fungicidal values for the same species and strain vary, some facts stand out distinctly: (1) a concentration of 1:100 killed[1] all fungi tested; (2) a concentration of 1:1000,000 did not effect any; (3) within the range of 1:100 to 1:100,000 the various fungi differ in their degree of sensitivity to clavacin; (4) M. albicans manifests the lowest degree of sensitivity and M. audouini the highest. T. gypseum and T. purpureum take an intermediate position; the latter is more sensitive than the former.

COMMENT

The fungicidal effect of clavacin was in direct proportion to the concentration of clavacin and to the length of time of its action upon the fungous elements.

[1] The terms "fungicidal", "lethal" and "killed" are arbitrarily used in this paper to denote lack of growth on a nutrient medium, of the exposed fungous material, after 21 days of observation.

Similar effects could be produced by a high concentration after a brief exposure and a low concentration after a long exposure. For instance, M. audouini was killed by a 1% solution after a 15 minute exposure and by a 0.01% solution after an exposure ranging from 1 hour to 48 hours. This rule operated only within certain limits. Higher dilutions failed to exert fungicidal effects.

A comparison of the fungicidal values of a 0.1% solution of clavacin revealed a difference in the response of the dermatophytes. M. albicans was not killed.

TABLE 1

TYPE OF FUNGUS	M. ALBICANS	T. GYPSEUM	T. PURPUREUM	M. AUDOUINI
Time required for fungicidal action....	0	48 hrs.	1½ hrs.	1 hr.

Fungicidal effect of undiluted culture filtrate.
0 = no fungicidal effect.

TABLE 2

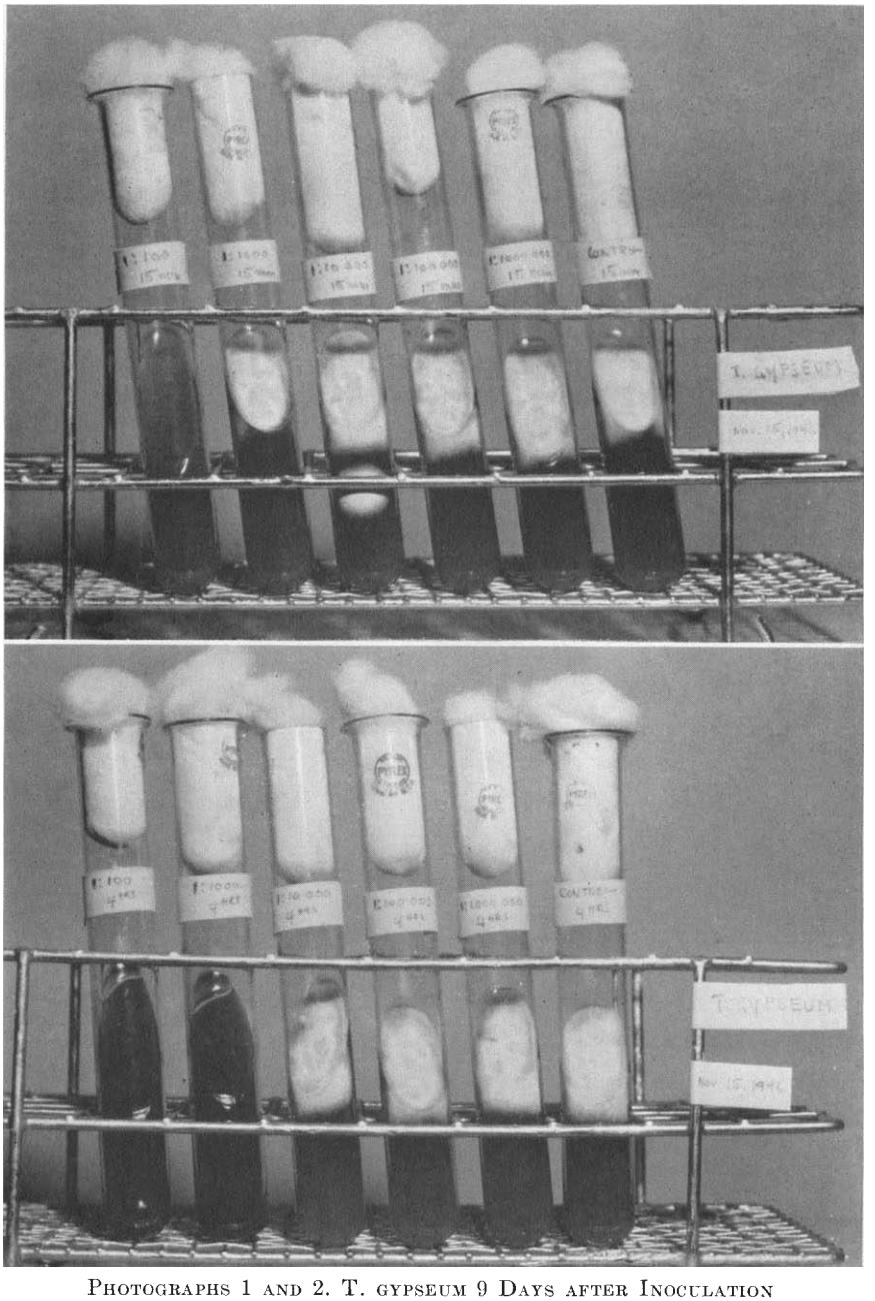
	1:100 (1%)	1:1000 (0.1%)	1:10 000 (0.01%)	1:100 000 (0.001%)	1:1000 000 (0.0001%)
M. albicans	24 hrs.	0	0	0	0
T. gypseum	3 hrs.	24 hrs.	0	0	0
	30 min.	3½ hrs.	0	0	0
	15 min.	15 min.	4 hrs.	48 hrs.	0
	15 min.	3 hrs.	0	0	0
T. purpureum	15 min.	2 hrs.	0	0	0
	15 min.	2 hrs.	48 hrs.	0	0
M. audouini	15 min.	15 min.	1 hr.	3½ hrs.	0
	15 min.	30 min.	24 hrs.	0	0
	15 min.	3 hrs.	0	0	0
	15 min.	2 hrs.	48 hrs.	48 hrs.	0

Time required for fungicidal action of various concentrations of crystalline clavacin. The data include the results of several assays.
0 = no fungicidal action.

T. gypseum was killed after exposures ranging from 15 minutes to 24 hours, T. purpureum consistently after 2 hours, and M. audouini after exposures of 15 minutes to 3 hours (see photographs 1–4).

The order of sensitivity to clavacin, in spite of greater individual variations, remained identical with that established in our preliminary report. Furthermore, a comparison of the data in tables 1 and 2 reveals that there is a similar relative sensitivity of the test fungi to both the culture filtrate and the crystalline clavacin.

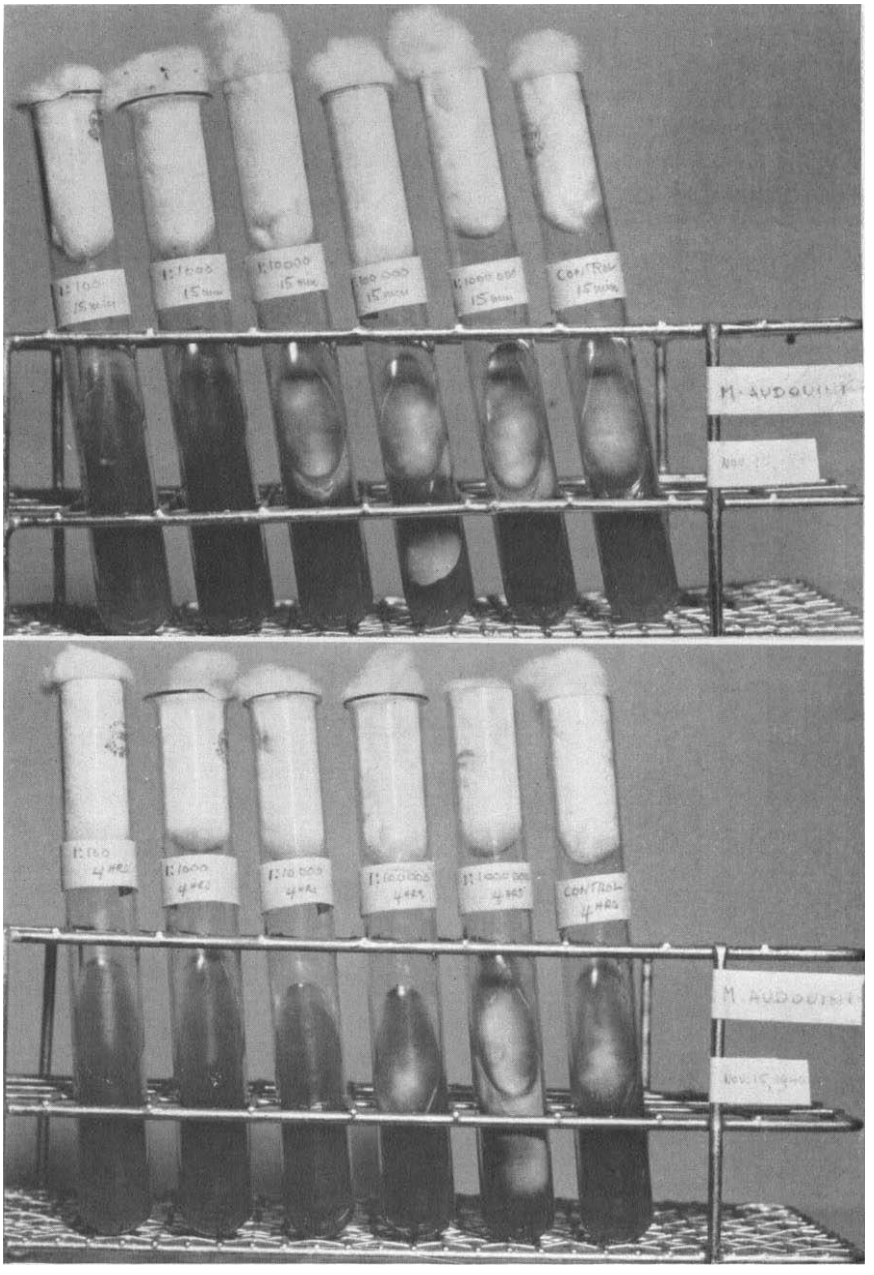
It is interesting to note that those species which are notorious for their resistance to therapeutic agents possess a relatively high degree of sensitivity to clavacin.



PHOTOGRAPHS 1 AND 2. T. GYPSEUM 9 DAYS AFTER INOCULATION

Fifteen minutes' exposure inhibited growth only in concentration 1:100. Four hours' exposure inhibited growth in concentrations 1:100 and 1:1000. Compare these data with those on photographs 3 and 4.

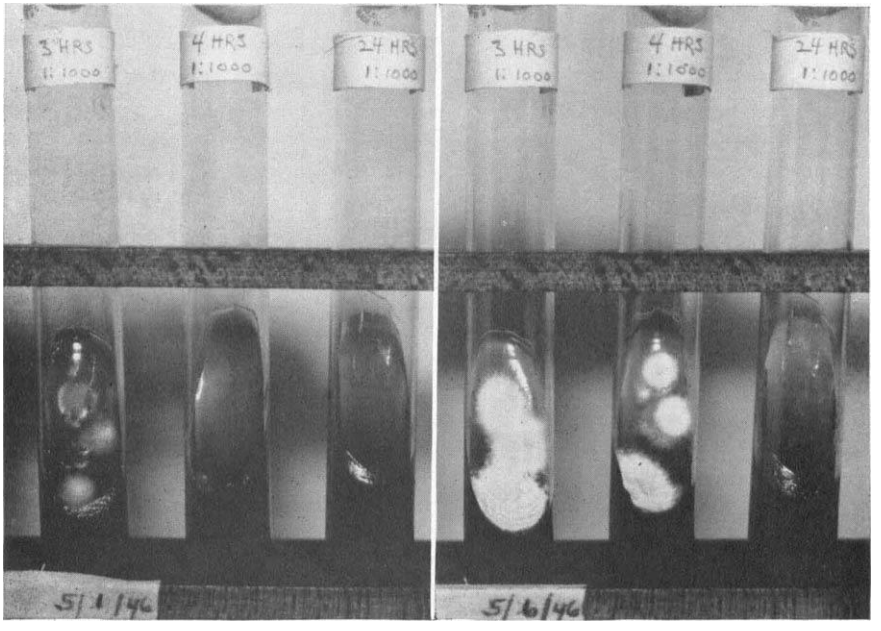
Further analysis shows that when concentrations too weak to produce a lethal effect were used, there was a retardation of growth of the test fungi. Too brief



PHOTOGRAPHS 3 AND 4. M. AUDOUINI 9 DAYS AFTER INOCULATION

Fifteen minutes' exposure inhibited growth in concentrations 1:100 and 1:1000. Four hours' exposure inhibited growth in concentrations 1:100, 1:1000, and 1:10,000. Compare these data with those on photographs 1 and 2.

exposures also retarded their growth. This delaying action was not noticeable with M. albicans. Photograph 5 illustrates the retardation of growth of T.



A. Nine days after inoculation. B. Fourteen days after inoculation.

PHOTOGRAPH 5. THIS ILLUSTRATES THE RETARDING EFFECT OF A 0.1% SOLUTION OF CLAVACIN UPON T. GYPSEUM, AFTER 3, 4 AND 24 HOURS' EXPOSURE RESPECTIVELY

TABLE 3

7 DAYS INCUBATION		21 DAYS INCUBATION	
Clavacin 0.1%	Control	Clavacin 0.1%	Control
0	++	++	++++

Retarding effect of 0.1% solution of crystalline clavacin upon T. purpureum, after 30 minutes' exposure.

TABLE 4

7 DAYS INCUBATION		21 DAYS INCUBATION	
Culture Filtrate	Control	Culture Filtrate	Control
0	++	+	++++

Retarding effect of undiluted culture filtrate upon M. audouini, after 30 minutes exposure.

Explanation of symbols for table 3 and 4.

0 = no growth; + = beginning growth; ++ = colonies covering up to one half of the surface; +++ = colonies covering major part of surface; ++++ = colonies covering entire surface.

gypseum by a 0.1% solution of clavacin. Four hours of exposure suppressed growth for nine days, but five days later the inhibitory effect was overcome.

Table 3 shows the retarding effect of a 0.1% solution of clavacin upon T. purpureum, after a 30-minute exposure. Seven days after inoculation no evidence of growth was present while the control colonies covered half of the surface of the slant. Twenty-one days after inoculation the exposed fungus had grown, but had attained only half the size of the control colonies. Table 4 shows that a similar delaying and stunting action was exerted by the culture filtrate upon M. audouini after an exposure time of 30 minutes.

SUMMARY AND CONCLUSIONS

1. Crystalline clavacin and a culture filtrate of Aspergillus clavatus revealed antibiotic action, in vitro, against the following dermatophytes: M. albicans, T. gypseum, T. purpureum, and M. audouini.

2. The fungi were killed by high concentrations of clavacin and long exposures.

3. A retarding effect was produced by weaker concentrations and short exposures.

4. The antibiotic effect varied with the type of fungus. M. albicans showed a high degree of resistance, while M. audouini was highly sensitive. T. gypseum and T. purpureum took an intermediate position, the latter being more sensitive than the former.

5. The test fungi showed a similar relative susceptibility to both the culture filtrate and the crystalline clavacin.

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